Support from the NIH NS053493 and the Dreyfus Foundation is gratefully acknowledged.
[1] Howard, Hudspeth, and Vale, Nature 342, 154–158 (1989).
[2] The Brownian motion of 2 μm beads in dilute PolymerX solutions and of 0.2–2 μm unattached vesicles in live cells, when analyzed by the Generalized Stokes-Einstein method, show similar G′ and G″ in the two environments.

704-Pos Board B583
Quantum-dot Assisted Characterization Of Helical Motor Paths On Microtubules
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Advanced techniques in single-molecule optical microscopy have contributed greatly to our current view on the dynamics of motor proteins. While so far most studies have been limited to the 2-D imaging on a CCD-camera chip, a complete understanding of motor protein function requires insight in how motor proteins move in 3-D on the lattice of cytoskeletal filaments.
Here, we report a novel and versatile method to study the interactions of motor proteins with cytoskeletal filaments in 3-D with nanometer accuracy. We sparsely label reconstituted microtubules with quantum dots and use fluorescence microscopy to image their longitudinal and rotational movement over reflective silicon surfaces coated with motor proteins. We determine the 2-D xy-positions of the QDs with sub-pixel accuracy by nanometer tracking and combine this data with simultaneous height measurements based on fluorescence-interference contrast microscopy. We use this technique (i) to investigate the stability of the paths of cooperating processive kinesin-1 motors and (ii) to study the asymmetry in the powerstrokes of non-processive microtubule motors.

705-Pos Board B584
Diffusive Movement Of A Processive Kinesin On Microtubules
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Conventional kinesin-1 is a processive motor protein that moves unidirectionally on microtubules. We found that when full-length kinesin containing a HIS tag at its C-terminus is bound to an anti-HIS Quantum dot (Qdot), it shows diffusive movement on microtubules in the presence of either ATP or ADP. Diffusive behavior was first described for the depolymerizing kinesin-13, MCAK (Helenius et al., 2006). When bound to a carboxylated Qdot, the same kinesin construct moves processively in the presence of ATP, but does not interact with microtubules in ADP. Further investigation with a truncated construct lacking the last 75 amino acids (kinesin-ΔC) showed both unidirectional and diffusive movement on microtubules in solutions containing a mixture of ADP and ATP. The diffusion constant depends on the concentration of ADP/ATP. When tested in solutions containing only ADP, kinesin-ΔC shows purely diffusive movement. We interpret these data to imply that kinesin-1 diffuses on microtubules when it is in the inactive, folded conformation, and it moves processively when in its active, extended conformation. We speculate that in the folded state, kinesin with bound ADP retains a relatively high binding affinity for microtubules compared to extended kinesin, thus allowing it to diffuse.

706-Pos Board B585
Alternating Site Mechanism Of Kinesin-1 Characterized By Single-molecule FRET Of Fluorescent ATP Analogues
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Kinesin-1 motor proteins move along microtubules in repetitive steps of 8 nm at the expense of ATP. To determine nucleotide dwell times during these processive runs, we are using here a FRET method at the single-molecule level that detects nucleotide binding to kinesin motor heads. We show that the fluorescent ATP analogue used produces processive motility with kinetic parameters altered less than two and a half-fold compared to normal ATP. Using our confocal fluorescence kinesin motility assay, we obtain fluorescence intensity time traces that are analyzed using autocorrelation techniques, yielding a time resolution of about a millisecond for the intensity fluctuations due to fluorescent ATP binding and release. To compare these experimental autocorrelation curves to kinetic models, we use Monte-Carlo simulations. We find that the experimental data can only be described satisfactory on the basis of models assuming an alternating site mechanism, thus supporting the view that kinesin's two motor domains hydrolyze ATP and step in a sequential way.

707-Pos Board B586
Expression and Characterization of Novel Rice Kinesin E15
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Kinesin is an ATP driven motor protein that moves along microtubules. Kinesin plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics, and signal transduction. Kinesin species derived from vertebrates have been well characterized. In contrast, only a few kinesis have been characterized in plants. E15 is one of the kinesin encoded on rice genome. E15 has COOH-terminal motor domain and exhibits a high homology with the kinesin-14 family in *Arabidopsis thaliana*. However, this kinesin is not similar to other kinesin -14 family kinesins derived from animal, e.g., DmNcd, ScKar3, and CeKlp. Consequently, kinesin E15 may be plant-specific kinesin. In this study, we expressed the motor domain of a novel rice plant-specific kinesin, E15, in *Escherichia coli*. and studied its enzymatic characteristics and compared with other related kinesins. Molecular weight of the E15 motor domain was 37.6 kD. The MT-dependent ATPase activity was higher and the affinity for MT was weaker than rice kinesin K16 that we have previously reported. The optimum pH was pH 6.0–6.5, which is similar to K16. Interaction of E15 with fluorescent ATP analogues was also studied for the kinetic characterization. E15 showed weaker affinity for nucleotide than other kinesins. Currently, we are preparing E15 dimer for the motility assay.

708-Pos Board B587
Kinetic characterization of the Rice Kinesins using Fluorescent-ATP Analogue
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Kinesin is an ATP driven motor protein that plays important physiological roles in intracellular transport, mitosis and meiosis, control of microtubule dynamics and signal transduction. Kinesins derived from vertebrate have been well studied on their characterization. However, not so many studies for kinesins of plants have been done yet. Previously, we have expressed the novel rice kinesin K16 by *E.coli*. Biochemical and crystallographic studies of the K16 motor domain demonstrated that K16 has very unique properties and conformation, which may reflect the plant specific physiological role. We have also succeeded to express other several rice kinesins. In this study, we focused on rice specific kinesins D04, L05, N14 and O12. The kinesin motor domains of D04 and L05 are found at the N-terminal. In our preliminary study, D04 and L05 belong to kinesin-4 sub family and kinesin-7 (CENP-E) sub family, respectively. On the other hand, N14 and O12 are the C-terminal motor domain. N14 and O12 belong to kinesin-14 family. Kinetic characterizations of these kinesin motor domains were studied using fluorescent ATP analogue, NBD-ATP. The binding of NBD-ATP to the ATPase site and release from the site were monitored by the change of fluorescence intensity. The kinetic parameters of rice kinesins were compared with other related kinesins. The kinetic parameters of rice kinesins were apparently different from that of conventional kinesin.

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Analysis of Crystal Structure and Solution Structure of the Motor Domain of Rice Kinesin K16
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The novel rice plant specific kinesin K16 has several unique enzymatic characteristics comparing with conventional kinesin. The most interesting property is that the ADP-free K16 motor domain is very stable, contrast to conventional kinesin that is very labile in ADP-free state. Recently, we have determined crystal structure of the novel rice kinesin K16 motor domain (K16MD) in complex with MgADP at 2.5A resolution. The overall structure of the K16MD is similar to that of conventional kinesin motor domains, as expected from the high similarity of amino acid sequence (43.2 %). However, the neck-linker of the ADP bound K16 motor domain showed an ordered conformation in a position quite different from that observed in conventional kinesin, which may reflect the unique enzymatic characteristics of rice kinesin K16. In the present study, we analyzed the inner structure of the K16 motor domain in detail and compared the structure with Eg5 and other related kinesins. It has been revealed that K16MD does not have interaction of amino acids side chains, which stabilizes the docking conformation of neck-linker. We have also analyzed the conformation of neck-linker in the solution using the K16 by FRET. Motor domain